Practical Implications for the Development and Deployment of Engineered Biosensors in Olive Oil Production

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Forward

UC Davis is located in California's agriculturally rich Central Valley and has a strong tradition of research at the complex interface between new technology, agriculture, and the maintenance of food safety and quality. The 2014 iGEM team sought to build on this tradition and to work on a project that could be considered uniquely "UC Davis". In collaboration with colleagues at the UC Davis Olive Oil Center we learned about the large-scale sale and mislabeling of rancid, sub-standard olive oil products and the need for inexpensive, reliable, and fast sensors for monitoring the quality of oil at all steps in the production process. It sounded like the makings of a great iGEM biosensor project.

However, we quickly discovered that the shape of any solution to our "client's" problem would need to be shaped by the needs of numerous stakeholders including lawmakers in Sacramento, farmers and producers, buyers for grocers and restaurants, and individual customers if it was going to stand a chance of being useful.

In the following we describe how our project was shaped by policy (in particular recent efforts to legislate olive oil quality in the State of California) and our various other stakeholder's practices. We submit this report to satisfy the iGEM competition's Gold Medal requirements. Since beyond-the-bench issues are common and critical to the practical implementation of nearly all iGEM biosensor projects - which often make up a significant portion of projects at iGEM - we hope that formally publishing our own analysis of the policy and practices issues we encountered will provide future teams with a perspective of how one team studied this issue and used what it learned to shape their project.

Section I
Project at a Glance
(context for this report)

- Approximately 70% of commercially available olive oil is detective due to rancidity or adulteration but is labelled as fresh - a possible ethical violation of consumer rights.

Figure 1: 70% of commercially available olive oil can be considered rancid.

- Olive oil exposed to oxidation, heat, or sunlight becomes rancid. Rancid olive oil fails to provide important health benefits.
- Olive oil producers and distributors may therefore benefit from an inexpensive and rapid means of detecting rancid olive oil.
- California has established new state standards to better its quality of olive oil and to establish a marketable reputation for quality.
- An inexpensive enzyme-mediated electrode biosensor was developed to detect a profile of aldehydes indicative of rancidity in olive oil.
- This reports describes the policy and stakeholder practices issues that guided the development of the biosensor.

Introduction to the Project

In response to the widespread sale of rancid and mislabeled olive oil, we sought to answer the following questions:

- Could an inexpensive yet robust biosensor be developed to detect aldehydes (chemical indicators of rancidity) in olive oil?
- If so, what sector(s) of the olive oil industry would benefit from the device and what design features would make it most likely to be adopted by one or more stakeholders?
- If this device (or something similar) were to be adopted, what might be some of the implications of its use on industry and consumers.

We began by identifying and gathering information from key stakeholders in the olive oil industry, including producers, millers, research scientists, and state regulators.

Figure 2: Identifying key stakeholders. Stakeholders included: (A) legislators, (B) olive oil producers, (C) consumers and (not shown) buyers (stores).

Based on our analysis of stakeholder needs in the context of iGEM's numerous logistical and financial constraints we identified and evaluated multiple possible concepts that we could potentially pursue.

Device concepts included two devices categories:

- cell-based biosensors (popular among previous iGEM projects), and
- electrochemical devices

Stakeholder constraints included:

- inexpensive price per test,
- rapid detection,
- quantitative output,
- unambiguous decision-making power,
- easy assembly and use,
- portable and robust enough to be used in the field, and
- an ability to identify specific compounds at low concentrations.

This led to the development of a multi-enzyme-linked electrochemical biosensor that met nearly all of the aforementioned criteria expect that (a) it is not yet able to detect aldehydes at sufficiently low concentrations and (b) a protective casing needs to be built before it can be deployed in the field. While our current device is not yet field ready we demonstrated a proof-of-concept success for each of the technical elements required to make our device real and expect that the additional engineering refinements are reasonable and feasible to achieve.

Purpose of this Report

In this paper we present a comprehensive review of the factors influencing this year's project, and the ways in which our project addressed these factors.

The paper is divided into several sections, each relating to our core question. At the beginning of each section, we discuss the relevance of the section and how the topic helped us better achieve our objectives. What follows is an in-depth exposition of the subject matter, meant to elucidate our rationale and provide insights into the context of the project.

Background

Health benefits of olive oil

Olive is an essential ingredient in the Mediterranean diet, and is world renowned for its taste and culinary versatility. However, few people appreciate the astonishing diversity of health benefits that olive oil offers. It is important for consumers to understand that when olive oil is adulterated or becomes rancid, these benefits are lost, and that this loss is significant. In this section, the benefits of olive oil are discussed, as well as the consequences of rancidity on the aforementioned health benefits. We emphasize that the loss of these advantageous properties is significant for consumer health, and that the question of whether a useful biosensor can be developed to address this situation is by consequence a valuable one.

Olives are a hugely diverse category of food with more than 40 species of olive trees and 700 varieties of olives grown around the world [1]. Consequently there are a myriad of olive oil varieties extracted from these olives. Each varietal has a unique flavor profile determined by its tree of origin, among other factors [2]. However, for commercial purposes, commodity olive oil is often classified into the following categories: pomace, refined, light-virgin, virgin, and extra virgin olive oil [2].

> *"Extra virgin olive oil is the oil that comes from the first pressing of the olives, and is considered the finest, having the freshest, fruitiest flavor,"*
> *- Dr. Timothy Harlan, 2011*

Bona fide extra-virgin olive oil is extracted from olives using only the application of mechanical pressure, a process known as cold pressing. Extra virgin olive oil (EVOO) is the least processed and is considered the most healthy category of olive oil. "It's the oil that comes from the first pressing of the olives, and is considered the finest, having the freshest, fruitiest flavor," says Timothy Harlan, MD author of *Just Tell Me What To Eat!* and assistant professor of clinical medicine at Tulane University [3].

Extra virgin olive oil contains numerous beneficial compounds including monounsaturated fats, polyphenols, and antioxidants. In fact, the plethora of active compounds in extra virgin olive oil have been found to improve digestive tract and cardiovascular function, and to provide anti-inflammatory, anti-aging, and even anti-cancer properties. Weinbrenner, et. al (2004), found lower DNA oxidative damage in healthy subjects exposed to a high phenol compared to subject exposed to a low phenol diet. They concluded that this might partly explain the lower incidence of cancer and cardiovascular disease in the Mediterranean area where consumption of phenol-rich extra virgin olive oil is high [4].

Ongoing research suggests that the compounds in olive oil may also mitigate active cancers. Pampaloni et al (2014) identified the phenolic compounds hydroxytyrosol, secoiridoids, and lignans as majorly represented, beneficial compounds in olive oil, demonstrating that these compounds had an in vitro antiproliferative effect on a colon cancer cell line [5]. Likewise, Coccia et. al (2014) demonstrated in vitro that human transitional carcinoma bladder cells showed a lower tendency to migrate through a metalloproteinase matrix when exposed to a high phenol environment [6]. They suggested that these phenols may also limit metastasis on human bladder cancers. Using a crude extract from extra virgin olive oil (EVOO), J. Menendez et. al (2011), showed in vitro that human breast cancer cell resistance to existing chemotherapy was reduced when exposed to the EVOO extract. They concluded that polyphenols found in EVOO could represent a valuable "pharmacologically active second-generation anti-cancer molecule" with a novel mode of action [7].

With regard to anti-inflammatory agents, it is believed that elevated levels of high density lipoprotein cholesterol (HDL-C) have protective, anti-inflammatory properties. Marrugat et. al Consumption of phenol rich virgin olive oils have resulted in increases in circulating HDL-C ranging between 5.1–6.7% in two human studies. Marrugat et. al linked increased phenolic virgin olive oil consumption to a decrease in TC to HDL-C ratio [8]. A reduced risk of all-cause mortality, cardiovascular disease, and stroke were associated with higher intakes of olive oil by Schwingshackl and Hoffmann [9]. Lastly, regular consumption of olive oil has also been linked to decreased blood pressure and lower levels of low density lipoprotein cholesterol (LDL-C), yielding a reduced risk of atherosclerosis and cardiovascular disease (CVD) [8].

As described above, extra virgin olive oil provides consumers with a blend of compounds offering improved metabolic, immune system, and physiological function. However, these health benefits are largely contingent upon the quality of the olive oil. The most important conditions contributing to the healthiness of olive oil are its being extra virgin, unadulterated, and in particular, not rancid [1]. Even EVOO will cease to provide health benefits once it has undergone oxidation, heating, or sun exposure. At that point, many of these useful compounds have degraded, rendering them functionally inactive. This is what the olive industry refers to as rancid olive oil [10].

In summary, fresh olive oil offers many important health benefits. However, these benefits are lost when the olive becomes rancid or is adulterated. In our research, we discovered that rancidity and adulteration is a serious problem in the olive oil industry. The widespread issue is discussed below, demonstrating the scope of our project.

The Rancidity and Adulteration Problem

In July, 2010, a study conducted by the UC Davis Olive Center found that approximately 70% of olive oils imported into the United States were rancid or otherwise defective due to poor handling, inadequate storage conditions that lead to oxidation and exposure to heat or light, as well as by the deliberate addition of non-beneficial, extraneous oils [11].

“Many olive oils claim to be virgin, extra-virgin, or light extra-virgin, but they don't in fact meet the standards of a true extra-virgin olive oil.” -Ruth Mercurio, 2014

In an article entitled “Food or Fraud?”, the Chemical and Engineering News Magazine (CEN) proclaimed extra virgin olive oil to be one of the five most widely adulterated food products on the market [12]. This adulteration may involve the spiking of olive oil with less-valuable canola oil, or the addition of poor quality or even rancid olive oil to extra virgin olive oil. In both instances, filling a desired volume with cheaper oil increases a profit margin.

Given the critical importance of olive oil quality in providing health benefits, it follows that close to 70% of the olive oils imported into the United States lack the health benefits promised to consumers. Provided that an estimated 96.6% of olive oil consumed in the United States is imported, this discrepancy in quality affects many people and a large industry [13]. However, beyond the U.S., many other countries import the majority of their olive oil as well. Defective olive oil is a global problem, not simply a regional problem.

As recent reports have shown, most people are not aware of this issue. According to Ruth Mercurio, a board member of the California Olive Oil Council (COOC), “Many olive oils claim to be virgin, extra-virgin, or light extra-virgin, but they don't in fact meet the standards of a true extra-virgin olive oil” [13]. There are a number of reasons for this incongruence. As mentioned previously, leading causes of defective olive oil include poor handling of olive fruit, inadequate storage, oxidative exposure, and excessive shelf life. The first three reasons are closely related. As is the practice in many countries, olives are outsourced from distance groves, are processed in a different country, and are then bottled in a country with a marketable name [10]. So a single bottle of olive oil may have its origins in multiple countries, introducing significant opportunity for poor handling and storage, particularly in countries with less developed infrastructure. As COOC board member Mercurio explains, if a bottle has a label that reads "Packaged in [name of a country]" it’s more than likely that the oil wasn’t grown

in that country, just bottled there to give it more cachet …" [13]. Thus olive oil may become rancid or degraded before it is even exported.

This leads to the fourth source of bad olive oil: excessive shelf time.

"If there's no harvest date on the label, you run the risk of purchasing an old, possibly rancid oil." That's because olive oil is not like wine: age does not enhance quality. "True EVOO has a shelf life of only 18-24 months" [13]. Even if imported oils carry a harvest date, retailers may keep olive oil on the shelf for well after the so called "time of minimum durability" (TMO), after which the olive oil has lost the organoleptic properties defined on the label [14].

We learned from personnel at the UC Davis Olive Center as well as producers in the field that another factor in the dissemination of defective oil is the inability of middle-level distributors and retailers to quantitatively evaluate the condition of their stock. The most common current methods of evaluating olive oil quality include gas chromatography (GC) and mass spectroscopy (MS). However, these methods are expensive and largely preclude the participation of olive oil distributors, retailers, and consumers in analytical quality control. From this information, we gathered that an inexpensive and widely accessible tool to augment current technologies would symbolize a step forward for the industry. However, tools like GC and MS are well-entrenched in industrial application, and will likely remain there do to their superior analytical capacities. However, this economic situation sparked our thinking. We surmised that a simple device capable of broad classification could be useful in situations where quantification is not necessary required, but rather a simple yes or no verdict.

"If there's no harvest date on the label, you run the risk of purchasing an old, possibly rancid oil."
-Ruth Mercurio, 2014

In summary, globally traded olive oil suffers from both widespread rancidity and adulteration. Poor handling, inadequate storage, excessive shelf time, and the blending of non-beneficial oils serve as leading causing in the propagation of defective oils. Rancid and adulterated olive oil does not offer the same important health benefits as extra virgin oil offers. The ability of these inferior products to saturate the global market is due in part to the inability of the olive oil industry to regulate quality at the distributor, retailer, and consumer levels. Likewise, permissive and unilateral standards contribute to the problem at hand, leading regional bodies like the California Department of Food and Agriculture to enact tougher regulations on produce.

The 2014 UC Davis iGEM team found this widespread issue a compelling problem to address in our summer research project. We believe that the deliberate sale of rancid or adulterated olive oil under the guise of extra virgin olive oil is a violation of consumer

rights. We believe that a product label should accurately reflect product contents and that regulatory bodies, producers, retailers, and consumers should have the tools to ensure the quality of their olive oil. As this paper delineates, we have constructed a tool with this motivation in mind.

Section II

Engaging Public Policy

Multinational Standards

A leading motivation for our project was the desire to develop a device that could bolster quality in the olive oil industry by cheaply detecting rancidity. In this project, we sought to knit to closely related questions together. We wanted to know 1. if we could make a device capable of cheaply detecting rancidity, and 2. if we were successful, who would use the device and how.

However, before we could tailor our project to meet the needs of the industry, we needed to learn how quality is defined within the industry. In particular, we wanted to better understand the ways in which regional and multinational organizations monitor and dictate quality standards. We also wanted to better understand how olive oil quality measures are put into practice, and how our device could be adapted to aid in this purpose. This pursuit of knowledge led us to meet with many stakeholders, from producers and millers, to trade representatives at the State Capitol. In the following section, we discuss the ways in which our project could help the California Department of Food and Agriculture (CDFA) maintain new California State standards on the quality of commercial olive oil.

The issue of food product adulteration and mislabeling is by no means new to the realm of United States legislation. Efforts to improve the quality of commodity and speciality food items date to the early 19th century, with varied degrees of efficacy. A watershed ruling came in 1911 when the Supreme Court, ruled in U.S. v. Johnson that the 1906 Food and Drugs Act prohibits false and misleading statements about the ingredients or identity of a product [15]. This principle applies directly to the contemporary issue of olive oil quality, providing a precedent for sanctioned prohibition of adulterated or mislabeled oil.

The de facto standards agency behind the olive oil industry is the International Olive Council (IOC), a global regulatory body established in 1959 by the United Nations to promote the “expansion of international trade in olive oil,” as well as the “drawing up and updating [of] product trade standards and improving quality” [16]. In fact, the vast majority, some 96% of the world’s nations, have become members of the IOC. Member nations may influence IOC policy, gaining representation proportional to their olive

production (see Figure 3 for top producers). However, to do so, members must legally comply with the regulations set forth by the Council.

Figure 3. *World Olive Oil Figures* International Olive Council, 2014

Production, Consumption, and Surplus Olive Oil in the World Average data 2006-2012 (in metric tons).			
Countries	Production	Consumption	Surplus
Spain	1,300,000	550,000	750,000
Italy	475,000	700,000	**-225,000**
Greece	320,000	235,000	85,000
Tunisia	157,000	36,000	121,000
Syria	152,000	114,000	38,000
Turkey	144,000	111,000	33,000
Morocco	106,000	80,000	26,000
Portugal	56,000	80,000	**-24,000**
Jordan	26,000	24,000	2,000
Data gathered in June 2013, from the International Olive Council, Asoliva, Ministry of Agriculture of Spain, Italy, Portugal.			

An ongoing difficulty for the olive oil industry is getting public policy to more tightly define what constitutes rancidity, adulteration, and fraud. According to Dan Flynn, executive director of the UC Davis Olive Center, “Importers assume that existing IOC standards have adequately guarded against fraud, which is clearly not the case” [13].

“Importers assume that existing IOC standards have adequately guarded against fraud, which is clearly not the case.”
-Dan Flynn, 2014

Though a non-member of the International Olive Council, (IOC), the United States has adopted the majority of IOC standards, under independent United States Department of Agriculture (USDA) authorization. Notably, the USDA provides inspection services to certify olive oils on a fee-for-service basis, but compliance with USDA standards is voluntary [11].

Not surprisingly, the lack of consistent, robust standards, and the recent surge in publicity regarding widespread adulteration and defective olive oil has prompted concerned regional policy makers to take action. The State of California has recently implemented a new set of standards that tighten regulations on commercial produced

olive oil, and that better reflect the high quality aspirations of Californian producers. The following discussion of the new standards explores the application of our biosensor in the Californian olive oil industry, and is intended to model a wider application of the technology to the global industry.

California State Standards

A core tenet of our project is that the ethical problem of defective and mislabeled olive oil is a global issue, not a regional issue. The desired solution is one that helps all stakeholders, domestic and international. Accordingly, we believe that the success of the California Department of Food and Agriculture (CDFA) in implementing olive oil standards is a step forward in the common effort of bettering global olive oil management and quality. By meeting with stakeholders in the regulatory realm, we came to understand that there is a growing interest in developing more rigorous quantitative standards for olive oil quality. Consequently, we understood that a quantitative device would be the focus of our project. To further guide our design process, we investigated the policy realm of olive oil legislation.

On July 15, we attended a CDFA organized a public hearing at the State Capitol in which record ed evidence and testimony was presented by olive growers, millers, and the general public on a set of standards proposed by the California Olive Oil Commission (COOC) entitled Grade and Labeling Standards for Olive oil, Refined-Olive Oil and Olive-Pomace Oil. The COOC is a organization of Californian olive growers and millers that represents their common interests [10].

On September 18th, 2014, the CDFA approved these standards, providing for improved labeling and testing standards for olive oil produced in California. These new standards were designed to place stricter regulations on the quality of virgin and extra virgin olive oil sold in California, and came into effect on Sept. 26, 2014 [10].

“Consumers and the trade need to understand the important quality difference between extra virgin/virgin olive oils … [the] California olive industry standard does this better than any of its many predecessors,” testified Paul Miller, president of the Australian Olive Association, at the public hearing in Sacramento on July 15th [13]. In the words of Dick Neilsen of McEvoy Ranch, “without better labeling standards, product grade standards and product testing, the ruse will continue” [13].

Perhaps the most important long-term effect of the new standards is the potential for a reputable, California olive oil brand-name to emerge on the global markets. According to Karen Ross, secretary of the state Department of Food and Agriculture "California agriculture has an enviable reputation for high-quality products sought by consumers here and around the world. We believe the time has come to designate a ‘California-grown’ olive oil, and these standards are an excellent way to do it” [13].

Brand-name recognition, a powerful driver of consumer opinion, may allow the Californian industry to clearly distinguish itself from the sale of inferior oils and engender consumer loyalty to a proven label. “The California olive industry will now be able to distinguish itself as the authentic, premium-quality, extra virgin olive oil producer to American consumers,” said Jeff Columbini, chair of the COOC. “Consumers will now be able to know that when they are purchasing and consuming California extra virgin olive oil, it truly is 100 percent extra virgin olive oil” [13].

After learning about the public interest in enhanced quality control, we decided to investigate how best our biosensor could be put to use in the field. That is, we wanted to understand the practicalities of implementing a device into the field, and to determine what elements within the olive oil industry would find the technology most applicable.

Section III

Project Applications

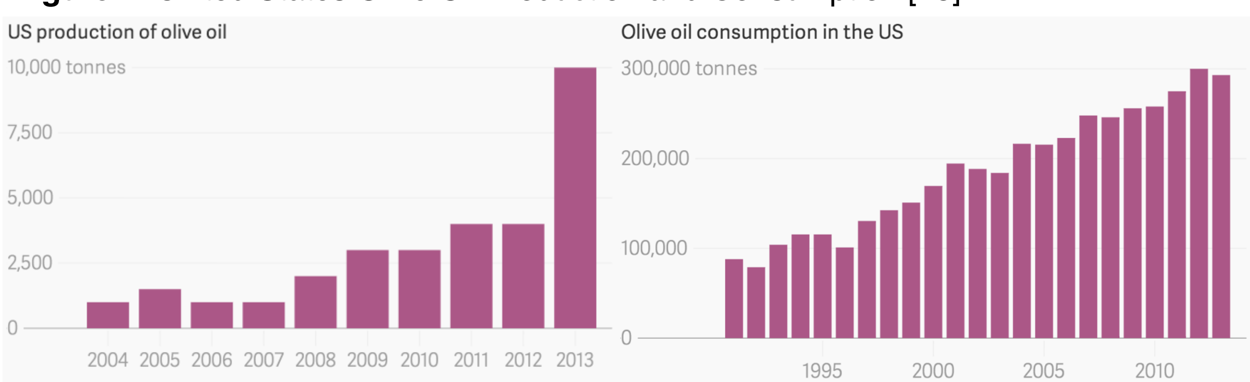
Technology Applications

As discussed below, the advent of new California State standards on olive oil quality could mark the emergence of a more uniformly high quality olive oil. After meeting with stakeholders in the industry, we found that the most important way we can help these standards succeed in improving olive oil quality is to empower producers, distributors, and retailers to monitor their stock individually.

Industrial Adaptation

American consumption of olive oil has grown meteorically in the past three decades, reaching 294,000 tons in 2014, a near three fold increase since 1990 (**Figure 4**). This places the United States as the third largest consumer of olive oil globally. Remarkably, only an estimated 3.4% of this oil is produced domestically [18].

Figure 4. United States Olive Oil Production and Consumption [18]



American olive oil production remains a nascent yet rapidly growing sector of the national food economy. With is Mediterranean climate and robust agricultural infrastructure, California helps lead this growth, increasing state production from 650,000 gallons to 870,000 gallons between 2008 and 2009, a 34% increase characteristic of the state industry [18].

According to David Garci-Aguirre, Manager of Production and Master Miller, Corto Olive Co., the quality control division of the olive oil industry is also growing in leaps and bounds, sparking new research projects within the field, and inviting the development of new quality assurance instruments to facilitate industrial scale-up [19]. During a June 2014 site tour of the Corto Olive Co. production facility, Mr. Garci-Aguirre expressed his interest in augmenting traditional and laborious methods of quality assurance, most notably gas chromatography, with more easily utilized and economical instruments. Other operations managers have corroborated this statement, offering future collaboration for testing of the prototype at their respective facilities.

Common to these managers was a belief that a rapid, cheap, and accurate biosensor for the detection of rancid compounds would be a useful addition to the olive oil industry. Thus, on the producer level, our enzymatic biosensor could be integrated into the division of quality control, allowing millers to more easily, cheaply, and thoroughly inspect the chemical profile of their products, and most importantly, make critical assessments as to whether their stock meets the stipulations of industry standards. This understanding of quality control is supported by a set of criteria delineated by the Food and Agriculture Organization (FAO) for quality control apparatuses. According to the FAO, quality control can be defined as “maintenance of quality at a level that satisfies the customer and that is economical to the producer or seller” [20].

Furthermore, the FAO outlines several variables nested within the subject of quality control that play important roles in the overall process. Several important topics include *product specification* (a written description of what the consumer is purchasing), *inspection* (the examination of a finished product to make sure it meets the specification), *process control*, (the insuring that all operations are performed in a way consistent with set standards) and lastly *quality”* [20]. Quite conceivably, our device fulfills these theoretical requirements of a quality control device, most notably *process control* and *inspection*. In practice, we envision the biosensor being integrated into multiple commercial stages. The device could test olive juice before official pressing to ensure that the olive fruits were not already fermented due to poor handling, during periodical, on-the-spot checks in the bottling process, and during distributor storage to identify rancid stock. Furthermore, retailers could periodically select random bottles from their shelves, test the oil for rancidity, and thus maintain high quality stock.

Naturally, the introduction of a device designed to insure objective quality evaluation could represent a disruptive force within the olive oil industry, allowing regulatory bodies like the California Olive Oil Commission to better enforce quality stipulations, and plausible exposing defective imports before they reach consumers. What follows is a review of the possible implications of our biosensor on the trade of olive oil.

Commercial Implications

The impact of food quality assurance has come increasingly into the forefront of public opinion. Concerns about food borne disease outbreaks and resulting human deaths, the use of hormones and antibiotics in food production, traceability of food products and the presence of genetically modified organisms (GMO) have entered public debate [21]. It is apparent that the quality of marketed olive oil in the US is very variable [11]. Health benefits that would be of interest to consumers are linked to virgin or extra virgin olive oil. Since most of the olive oil marketed as virgin or extra virgin olive oil is not of that quality, there is the potential for significant consumer concern if this information moved into the public arena.

In this section, we analyze the effect of our biosensor on the trade of olive oil and we frame the analysis with the context of the newly passed COOC standards, as a proxy for general quality control regulations. Existing and future legislation may broaden the olive oil standards to include imported olive oil and create the possibility for a California State seal for high quality (virgin and extra-virgin) olive oil. Two important topics discussed are the effect of olive oil quality control measures on domestic competitiveness and the impact of an olive oil quality seal on consumer decision-making and product price.

As noted in the COOC *California State Standards* in the introduction, tighter restrictions have already been placed on what can legally be labelled and sold as virgin and extra-virgin olive oil in California. For example, the benchmark for free fatty acid (FFA) content has now been set to 0.5 percent, lower than the more permissive international standard of 0.8 percent [10].

Proponents of the newly adopted standards for olive oil point to other California agricultural commodities that have benefited from enforceable standards. The California Farm Bureau Federation (CFBF) has stated that quality standards exist for 31 state commodities including almonds, pistachios, and walnuts, and that respective industries have "benefited over the long run from establishing strict quality standards for their respective commodities" [13]. Furthermore, the CFBF maintains that quality regulations "improve customer satisfaction by ensuring only high quality products are marketed". (These standards are available from the California Department of Food and Agriculture (CDFA) at cdfa.ca.gov). To that end, it is common knowledge that consumers have associated food quality with a number of positive attributes for many years. Steenkamp and Meulenberg found that perceived food quality is associated with "keepability, wholesomeness, appearance, well-known brands, taste, price, and nutritional value," attributes consistent with higher quality olive oil [22].

Accreditation processes have been found to engender both consumer confidence and loyalty. These processes, when linked to quality seals enable consumers to quickly identify superior goods in the market. Seal recognition promotes regional brand-name

loyalty and influences consumer decision-making. However, once a quality seal has been created and a standard established, it is imperative that the seal's reputation for quality be maintained. Maintenance of product quality is especially important "because a brand name becomes associated with a particular quality level, and any lowering of the level causes the customer to lose confidence in the brand; sales of other goods under the same brand may then also be reduced" [20].

Two Greek studies of consumer behavior by Duquenne and Vlontzos found that Greek households recognized certification as a guaranty of quality [23]. This recognition of certified quality translated into a significantly increased willingness to pay (WTP) for higher quality oil authenticated by the olive oil seals. Furthermore, in their study more than 56% of Greek consumers were disposed to pay a price at least 10% higher for certified high quality olive oil and 22.5% were willing to pay more than 20% more for a superior product [23].

If locally produced California olive oil was shown to be of the highest quality (extra virgin and virgin) and consequently also most likely to be linked to the health benefits, it is reasonable to suggest that if this superior product was recognized by a quality seal that consumers would purchase this product. In fact, in a recent olive oil lay press article there is already recognition that the new CA olive oil standards will be beneficial to domestic olive oil consumption. "The approval of the standards marks a victory for the fledgling California olive oil industry, which hopes that new testing and labeling requirements will provide a boost for locally-produced olive oil" [13]. Furthermore, any quality assurance programs implemented by the State of California is likely to convey a competitive advantage to domestic producers covered by the program. Brendahl and co-workers described this relationship in Europe; "a credible quality assurance system may reduce transaction costs, particularly the costs associated with searching and screening for suitable customers or suppliers, in negotiating the terms of a contract, and monitoring and in enforcing the terms of the contract" [24]. The United States Department of Agriculture likewise affirms that domestic standards may increase domestic competitiveness, noting that "Domestic customers' specifications may act to reduce the competitiveness of foreign suppliers" in favor of domestic producers meeting these specifications [25].

This increased in domestic competitiveness and regional market power may also be explained by fundamental consumer demand for superior goods. In a real market, consumers are faced with a variety of competing products and must choose the good the best meets their prerogatives. At a basic level, consumer desire to purchase higher quality product drives demand for superior products over inferior alternatives that are less capable of meeting their needs. In the case of olive oil, consumers would desire authentic extra virgin olive oil over lower quality, possibly suspect olive oil so as to meet a basic desire for health and taste benefits. Quality seals enable consumers to quickly and repeatedly identify superior goods in the market, developing customer loyalty and purchase patterns, and increasing sales for producers associated with the recognized label. Seal recognition is also important in standardizing regional brand

names, allowing members of a seal organization to associate their product with the reputation of a geographic region (like the Mediterranean region), conferring increased marketability to their product [26].

It is important to note that consumer decision making is also strongly linked to level of education, both in a professional or academical sense, but also in the context of how much information consumers are given about a product. As Lazarova notes: "Level of education plays also an important role in the information processing…less educated consumers will use fewer cues in the quality perception process and rely on cue information from personal sources rather than neutral sources of information" like chemical content and rancidity status. [27]. Likewise, according to the CDFA report on the new California olive oil grade and labeling standards, the "standards provide a foundation for educating consumers about olive oil grades" [10]. These standards will "ease consumer apprehensiveness toward olive oil products and help consumers make more informed purchasing decisions" [10]. As members of iGEM, we believe that educating the public through technology is an important investment. Providing a widely accessible device that elucidates olive oil content would allow consumers of all educational backgrounds to be make better informed decisions about the olive oil they purchase.

"Standards provide a foundation for educating consumers about olive oil grades."
- Rosica Lazarova

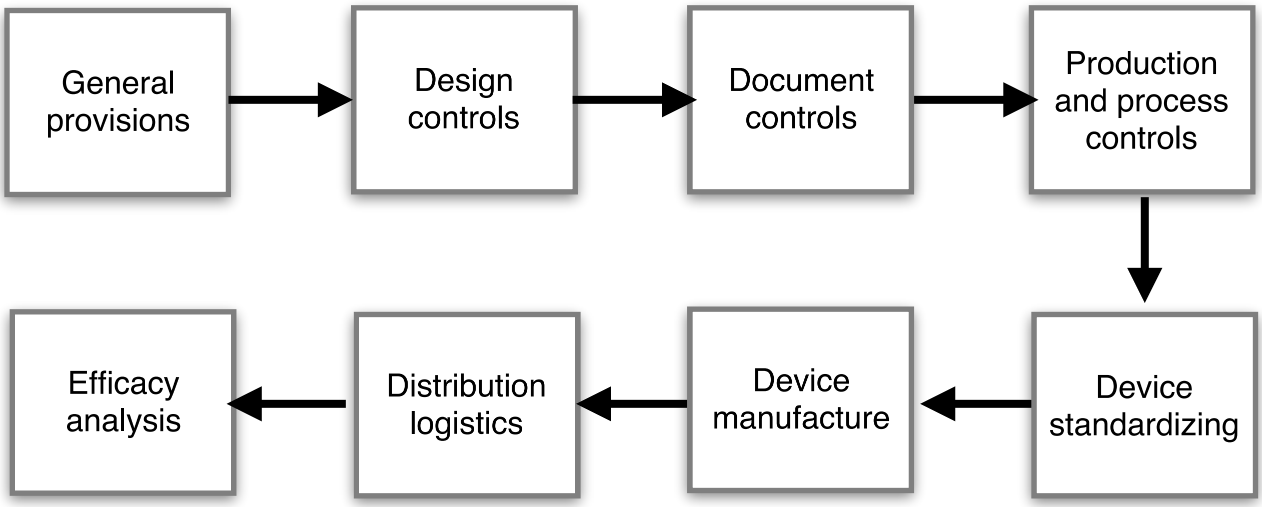
As mentioned before, rancidity is most likely to affect imported olive oil products since transport and storage times are key factors leading to rancidity. It is, of course, possible that any olive oil producer or processor could willfully adulterate olive oil with rancid olive oil or even canola oil. Producers who adulterate their olive oil, or who sell old and rancid stock do so to save money, because filling larger quotas with lower quality product is more product than filling small quotas with higher quality product. Notwithstanding, since California olive oil producers serving the domestic market will not face the significant international transport challenge they will be more likely to produce a high quality product into the market. Assuming that the biosensor technology will be able to detect rancidity in imported olive oil products, it is likely that the domestic producers will be able to differentiate their product on quality and thus gain market penetration.

In summary, if a low cost biosensor for detecting rancidity (aldehydes) in olive oil was readily available it is reasonable to assume that producers and retailers could use the device to classify their oil as rancid or not, and to thus clear the market of defective product. If that happens, the biosensor that we have developed could enhance the CFDA's ability to not only maintain a quality standard but augment the strength of a state quality seal. It therefore follows that our biosensor device would in part influence consumer purchasing patterns and ultimately the trade of olive oil.

In our project, we learned the importance of meeting with stakeholders in an industry, in our case olive oil producers, millers, and food service representatives, as well as the value of gathering meta-data on the potential application of a project in an industrial setting. Gathering testimonies from stakeholders in the industry is a valuable means of refining project design and establishing what the industry need really is, on a very practical level. We also learned that implementing a device into a quality assurance program is a lengthy and intensive process, requiring a proposed technology to meet many stipulations. On this basis, we concluded that the best avenue of project development would be to produce a device that would augment existing quality control measures, rather than supplant them. The most basic and required certification is the FDA certification, discussed below.

The FDA has established a Code of Federal Regulations (CFR) to oversee the implementation of quality control instrumentations in the health sector. CFR Title 21 pertains to medical and analytical devices (including biosensors), and is the relevant documentation pertaining to biosensor development and legal acceptance [28]. This system of quality regulations consists of 15 individual subparts that stipulate what criteria a biosensor device must comply with before it can be considered for authorized commercial implementation. These clauses are schematically summarized in Figure 9 below, and include, in part, the following: General Provisions (Scope, Definitions, and Quality System), Quality Audit, Personnel Training, Design Controls, Production and Process Controls, Process Validation, Inspection, Measuring and Equipment Testing, Device Labeling, Device Packaging, Installation, and Distribution. For a future iteration of our biosensor to be considered for industrial implementation, an operational protocol would need to be developed, meeting the criteria for the Process Control, Equipment Testing, and Process Validation subclauses, to name a few. Moreover, for entities involved in the distribution of analytical device like our own, yearly registration with the FDA is required. So proper market research would be required to guarantee that profits may out-weight these fixed costs. Further information on FDA standards can be found on their website, www.accessdata.fda.gov/scripts [28].

Figure 5. FDA Certification process for analytical devices



The FDA Code of Federal Regulations (CFR) Title 21 identifies 15 substeps in the analytical quality control device certification process. Each step requires a producer to identify a protocol by which to meet CFR requirements. These sub steps are summarized in eight steps above [28].

As mentioned above, the FDA is the primary, obligatory regulatory body through which aspiring technologies must be ratified. This, however, meets only basic requirements, and often customers seeking to purchase a quality control device look to products that have been certified by the International Organization of Standardization (ISO). ISO certification is voluntary, but highly regarded in the world of quality control methods [29]. ISO standards act indirectly upon a product, in that they do not offer topic specific regulations, like the California State standards are to olive oil. Instead, the ISO program simply certifies a product as having undergone the rigors of a comprehensive systems protocol. This protocol outlines all the important questions and considerations that must be addressed in creating a quality assurance product, and requires producers to thoroughly answer all these questions. ISO standards are designed to streamline manufacturing and ultimately to ensure the highest level of customer satisfaction. By complying with the ISO system, companies can differentiate themselves as customer-centric and dedicated to produced the highest value product. In our case, the ISO 9001:2008 protocol is the documentation most relevant to our biosensor [29].

This comprehensive undertaking highlights the fact that getting marketable certification is not a trivial matter; for our application, we believe that a non-certified device designed to assistance quality control would be the most appropriate solution.

Section IV

Project Overview

Project Design

After gathering information on the needs of industry stakeholders, and taking into consideration potential sectors of the olive oil industry that our biosensor could target, we began designing our device to meet specific criteria. The characteristics that this device was required to meet include: be inexpensive in price and rapid in detection so as to incentivize use, be accurate enough to give unambiguous decision-making power, be build of commercially available components for easy assembly and use, and to be portable and robust enough to be used in the field. As collaborators at the UC Davis Olive Center informed us, our biosensor would also need to identify specific aldehyde compounds within a low concentration target range. All these factors culminated in the design scheme of our biosensor.

After this foundation was established, some primary questions we sought to answer in our preliminary design were as follows: what chemical compound(s) to test for, what level of sensitivity was necessary, how to accurately detect and quantify these compounds, and how to make an inexpensive and portable device that performs well in varied environments.

Certain decision points were reached prior to in-depth project design. Up front, we decided against a cell-based system for two reasons. Primarily, we did not want to introduce a living (bacterial) systems into a quality control device that industry regulations on quality control would likely prohibit. Secondly, we did not want the complications of bacterial growth, culture stability, and containment issues that are associated with bacterial systems. From our tours of the olive oil mills, we learned that industrial quality control (QC) labs are often staffed by lower-skilled and sometimes temporary employees running numerous procedures between tight deadlines. Thus we chose to pursue the most simple system capable of achieving our objectives.

Collaborators at the UC Davis Olive Center informed us of that certain long chain aldehydes of both saturated and unsaturated are associated with rancidity in olive oil. Notably, these aldehydes are products of olive oil oxidation, and are highly objective indicators of rancidity found in all varietals and grades of olive oil [11].

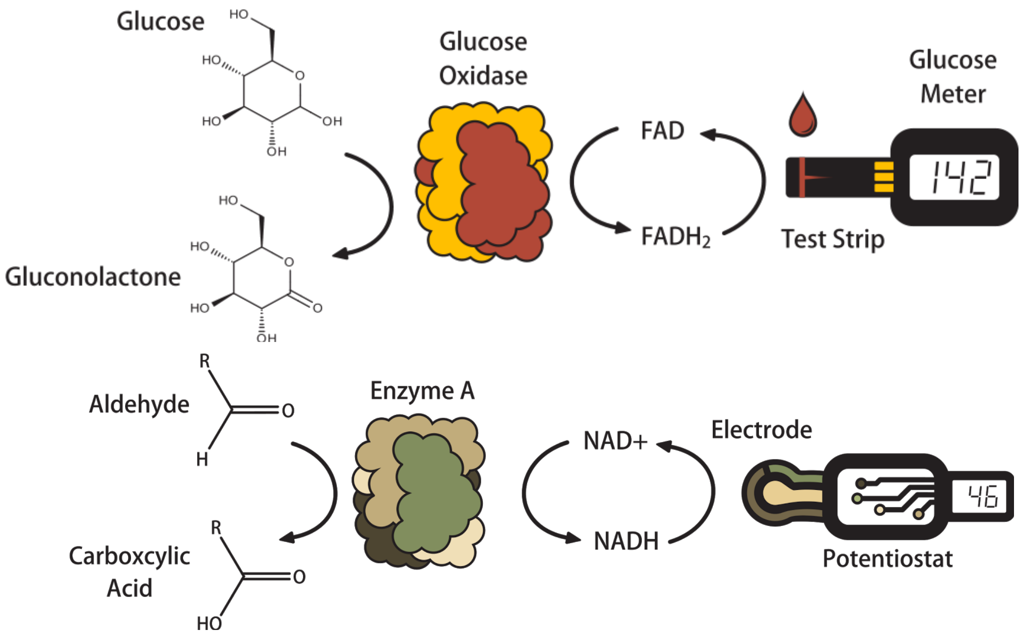
Figure 6. Major volatile compounds in rancid olive oil with determined concentration. Courtesy of UC Davis Olive Center.

Fresh Extra Virgin Olive Oil		Rancid Extra Virgin Olive Oil	
Volatile Compound	Concentration (μM)	Volatile Compound	Concentration (μM)
Pentanal	27.99	Hexanal	33.12
E-2-Pentenal	0.93	Nonanal	13.01
Hexenal	25.02	E-2-Octenal	38.08
E-2-Hexenal	227.03	E-2-Decenal	14.59
Nonanal	5.08	E,E-2,4-Heptadienal	19.93
E-2-Decenal	8.06	E, E-2,4-Decadienal	31.38

For this reason, we focussed on the detection of long-chain aldehydes over other compounds. However, many of these aldehydes are present in low concentrations, requiring fine sensitivity to verify their detection above competing background noise. Through a review of literature related to the quantification of low concentration analytes, we chose to develop an enzyme-mediated, electrode biosensor. Many applications of enzyme-electrode systems are reported in literature, from the detection of harmful pesticides to various medical applications. Common features include low concentration quantification, fast signal output, and inexpensive components [30].

A great example of an enzyme-mediated biosensor is the standard glucose meter used by millions of people worldwide to monitor blood glucose levels. This portable device utilizes the enzyme glucose oxidase, the cofactor FAD, and glucose to generate an electrical signal that the device interprets and reports as blood glucose concentration. In fact, we used the characteristics of a glucose meter to guide our own design process, understanding that the ergonomics and raw functionality of the device has made it such a success across the world.

Figure 7. Comparison of OliView to a standard glucose meter



A core hardware component of most enzyme-mediated biosensor is a transducer device known as a potentiostat. Potentiostats are widely used instruments in electrochemistry. They are essential to the correct application of voltage across a three electrode system, and can described as a integrated circuit containing simple operational amplifiers (op amps). However, potentiostats can cost well into the tens of thousands.

Not to be outdone by economics, we decided to build our own potentiostat for a fraction of the cost. This was easier said than done. However, several circuit board iterations and two thousand lines of code later, we had a fully operational sensor capable of measuring NADH to the micro-molar concentration, and aldehyde at 500 µM concentrations (see Figure 4 and Figure 5). Further optimization would focus on closing this gap.

Project Components

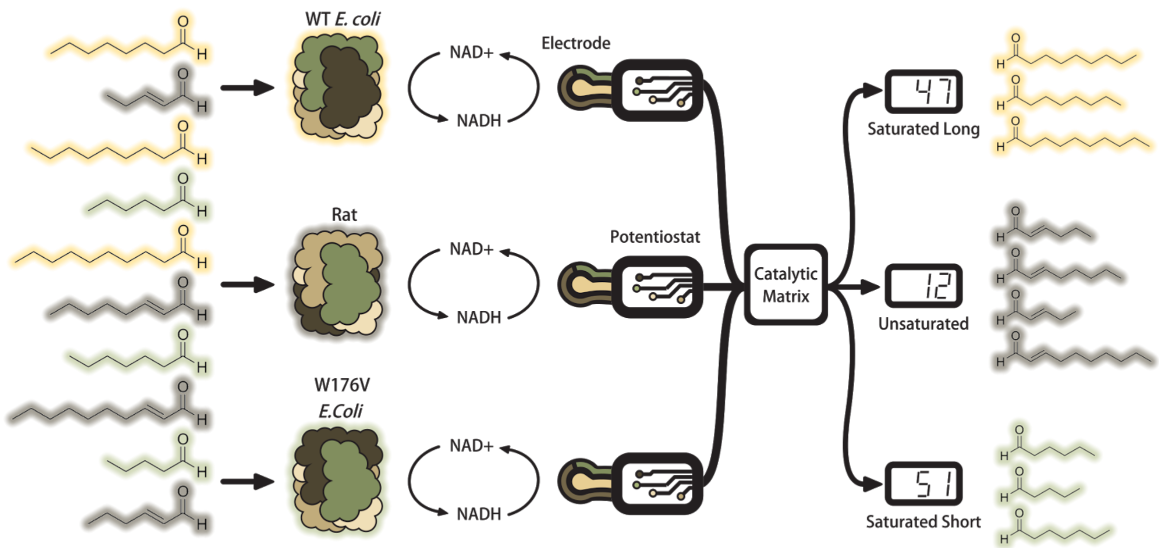
Our project consists of three modules: the enzyme solution, the electrode system and signal amplification system, and the software deconvolution system. Spatially, the apparatus works as follows. The enzyme solution is placed onto the electrode system, which measures the concentration of analyte, generating an electrical signal.This signal is amplified by the transducer circuitry, passed onto the computer program, and finally deconvoluted by the custom software. The computer output is the measured analyte concentration. Let’s examine each part individually.

The enzyme solution was buffered to maintain optimal conditions for the enzyme and its kinetics. The operant compounds in our solution include the aldehyde dehydrogenase enzyme, the cofactor NAD+, as well as aldehyde species. As shown in Figure 5 above, the overall system relays on the reduction-oxidation (redox) reactions of enzymes with substrate to generate an electrical signal. At the enzyme's active site, an aldehyde is simultaneously converted to a carboxylic acid while a cofactor like FAD or NAD+ is reduced to FADH2 or NADH, respectively. These reduced cofactors serve as electron shuttles, depositing acquired electrons onto the surface of the electrode.

The electrode system consists of a screen-printed chip embedded with three electrodes: the counter, working, and reference electrodes. Though the potential of the reference electrode is kept constant, a voltage bias is applied across the working and counter electrodes to facilitates a buildup of excess positive charge on the working electrode. This buildup induces the directional diffusion of NADH toward the working electrode. The working electrode consists of a carbon ink infused with a polycyclic aromatic monomer dye called Meldola's Blue (MB). We specifically ordered MB-infused electrodes from a company called Dropsens for the following reason. MB has a selective oxidative affinity for NADH, reducing the over potential necessary for NADH oxidation at the working electrode, and facilitating greater electron deposition and flow. The resulting current from the working electrode is then amplified by our custom-made potentiostat, an instrument designed to process and amplify electronic signals. Once processed, the signal is sent to a computer. The software then utilizes combinatorial linear algebra to output the measured concentration of analyte.

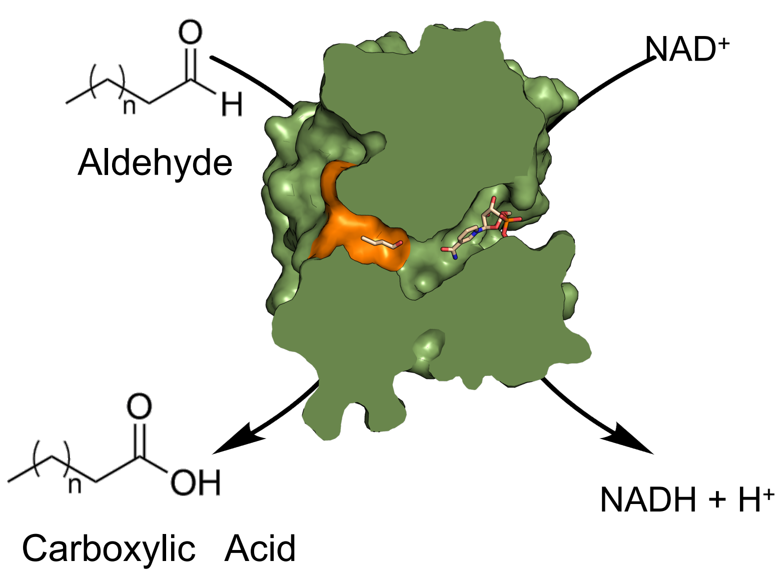
For our project, we decided a step beyond conventional biosensors. We decide to build a multiplexed biosensor in which not one but several rationally engineered enzymes react with specific analytes, generating numerous chemical profiles, rather than just one (Figure 8). This is crucial for the potential use of the biosensor in quality assurance programs, in which many types of organic compounds must be accurately quantified. In the case of olive oil, there are dozens of varieties of compounds that the California State Standards require testing for. Thus it is an important proof of concept to establish a simple multiplex design that can be expanded to detect a wider spectrum of compounds [10].

Figure 8. Multiplicity of an enzyme biosensor.



Multiple rationally engineered aldehyde dehydrogenase enzymes react with different aldehyde species. Aldehydes are converted to carboxylic acids, cofactor are reduced, and electrons deposited on the electrode to generate a current. This current is then translated by software into a reading.

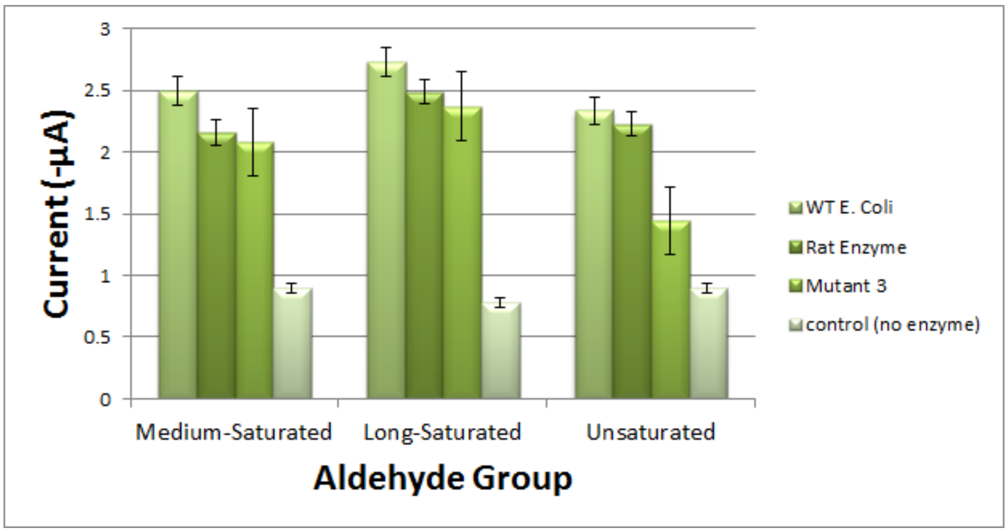
Figure 9. Structure and function of rationally engineered aldehyde dehydrogenase (ALDH)



Aldehyde dehydrogenase (ALDH) enzyme with internal active site; substrate and cofactor catalyzed in the enzyme "tunnel."

Several aldehyde dehydrogenase (ALDH) enzymes were engineered, each mutant originating from either an *E.coli* ALDH or a rat ALDH. As shown below in Figure 8, the rat ALDH demonstrated general specificity for all class aldehydes, while the successfully engineered ALDH demonstrated selectivity for only long chain (C6-C10), saturated aldehydes. Thus when a solution of aldehyde is tested on our biosensor with each enzyme individually, the results will either register as high signal for Rat ALDH and low current for the ALDH, or high signal for and low signal for Rat ALDH. This incorporates the idea of "binning" into our design, using two diametrically functionally enzymes to make broad distinctions.

Figure 10. Engineered enzyme specificity for both saturated and unsaturated aldehyde species

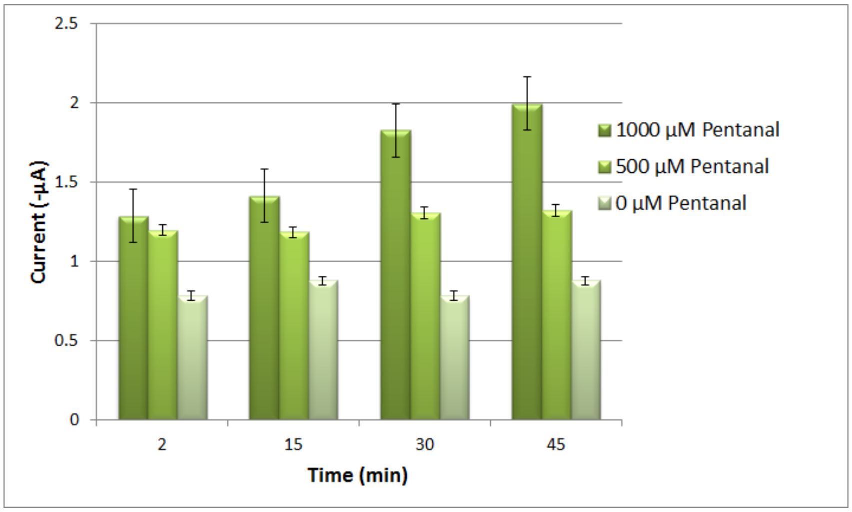


The rat aldehyde dehydrogenase (ALDH) demonstrated general specificity for all aldehyde species at 1000 µM, while the mutant *E. coli* enzyme demonstrates selectivity for unsaturated aldehydes 1000 µM.

The use of a third enzyme mutant, with specificity for short chain, saturated aldehydes, adds a third important distinction. When all three enzymes are tested with a sample of unknown aldehyde solution, the results test us whether the solution contains aldehyde of saturated or unsaturated form, as well as long or short chain length. This provides all the information we need to make a basic conclusion. If the solution contains aldehyde with long-chain length or unsaturated form, we can say with confidence that it is rancid. If the solution contains short-chain and not long-chain aldehydes, the solution is not shown to be rancid.

Though we were able to detect enzyme specificity at higher concentrations like 1 mM, our device had difficulty detecting enzyme activity at lower concentrations. As shown in the figure below, 1000 µM aldehyde yielded a clear increase in current over time, while 500 µM aldehyde did not. This indicated a need to enhance system sensitivity before lower range detection would be possible.

Figure 11. Limiting Affect of Substrate Concentration on Enzyme Activity



Aldehyde of 500µM concentration is shown not to register an increase in current over time. Thus the system requires further optimization before enzyme activity can be ascertained at 500µM and lower concentrations of substrate.

Significance of Project

Over the summer, we developed a working prototype of an enzyme-mediated biosensor. Several crucial proof of concepts were made, including the rational engineering of aldehyde specificity in aldehyde dehydrogenate, the construction of a $70, 16-bit Arduino compatible potentiostat with demonstrable similarity to professional potentiostats, and the successful differentiation of aldehyde compounds on the electrode system using the engineered enzymes. With continued development, these preliminary achievements may be expanded to refine system sensitivity and to feature a wider range of substrate specificity. This would enable the biosensor to detect more types of compounds in olive oil at lower concentrations.

Technology Application to iGEM

We are not the first iGEM team to develop a biosensor in the field of food quality. However, we might be the first team motivated in part by the inauguration of quality control standards, and by the desire to provide a cost-effective device to an industry. As listed below, past iGEM teams have constructed innovative projects in the field of food quality and consumer safety.

Bacterial Reporter Biosensors

- The 2013 ITB Indonesia team created a “whole cell” biosensor for aflatoxin B1 detection in food. *E. coli* was used as a chassis, utilizing a reporter gene and the color change of the bacteria to indicate the presence of aflatoxins.
- The 2013 University of Warsaw team also utilized a bacterial system, creating “FluoSafe,” a biosensor designed to detect acrylamide, a carcinogenic and neurotoxic compound found in fries and chips.

Bacterial Biosensors Augmented with Hardware

- The 2013 Sumbawagen team (from Indonesia) similarly created an *E. coli* biosensor capable of measuring the level of sugar in honey through the emittance of light from luciferase. According to their wiki, “Our final goal is to create a device which can be used for quality control of Sumbawa honey, which we call ‘ECONEY’.” They focussed on developing an electrical counterpart to their biological system.

- The 2013 TU_Darmstadt team “Hunting Fungi” describes their project objective as the development of a “handy device which allows an easy, fast and reliable detection of mycotoxins.” The design utilizes the conformational change of a TAR in response to mycotoxins, and ensuing emission of a “FRET-beacon” by flurophores. Their hardware includes a handheld-device linked to a controlling Smartphone App.

From what we have learned, we recommend that future iGEM teams desiring to build a quality assurance project should first consult the literature and regulatory contexts of their chosen field of application. It is important to understand from the beginning what technical and regulatory hurdles must be surmounted before successful implementation of the design may occur. This will guide the team in choosing the most appropriate technology solution, and to ensure that the project could meet the needs of the given industry, either in its present form, or after further development and iterations of the project. Likewise, remaining cognizant of the challenges of implementation may allow teams to form more realistic and attainable aspirations for their project. For instance, building robust proof of concept projects expands the body of experimental research within a field, laying the foundation either for continued development of that particular project, or for other researchers to build upon the work and to bring a new technology into the field.

Teams with the ambition to develop a food quality or consumer safety device may readily incorporate our technology platform into their project design. The inexpensive, open-source potentiostat biosensor we built for the detection of low concentration analytes may be adapted to many other biosensor applications that utilize electrode systems in their method of detection. Future teams utilizing our multiplex design will be equipped to rapidly detect low

concentration analytes in a complex solution with enhanced limits of detection and the ability for accurate quantification. Detailed instructions for assembling our potentiostat can be found free of charge on our team wiki page (http://2014.igem.org/Team:UC_Davis/Potentiostat_Design).

Conclusion

In summary, the UC Davis iGEM 2014 team has developed an enzyme-mediated electrode biosensor for the detection of rancid compounds in olive oil. The biosensor was designed to fulfill the criteria we established in our preliminary parameter analysis, and to meet the needs of olive oil producers and retailers as well.

In retrospect, our biosensor met all of the aforementioned criteria expect that 1. it was not able to sufficiently detect aldehydes in low target concentrations and 2. it requires protective encasing before it can be employed in the field. The results of our project indicates that the current technology is not yet able to fully answer the questions at hand. However, the successful creation and validation of an electrochemical biosensor utilizing enzymes suggests that this is a technology that could be refined and ultimately used in the olive oil industry.

It is our hope that future iGEM teams may benefit from our foundational technology, and incorporate our design into new projects within important fields relating to human health, like food quality and consumer safety. That way our project can leave an impactful legacy on the iGEM program, paving the way for future innovation.

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